Genetic polymorphisms of DNA repair genes and chromosomal damage in workers exposed to 1,3-butadiene

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The base excision repair (BER) pathway is important in repairing DNA damage incurred from occupational exposure to 1,3-butadiene (BD). This study examines the relationship between inherited polymorphisms of the BER pathway (x-ray repair cross-complementing group 1 (XRCC1) Arg194Trp, Arg280His, Arg399Glu, T-77C, ADPRT Val762Ala, MGMT Leu84Phe and APE1 Asp148Glu) and chromosomal damage in BD-exposed workers, using the cytokinesis-blocked (CB) micronucleus (MN) assay in peripheral lymphocytes of 166 workers occupationally exposed to BD and 41 non-exposed healthy individuals. The MN frequency of exposed workers (3.39 ± 2.42%) was higher than that of the non-exposed groups (1.48 ± 1.26%) (P < 0.01). Workers receiving greater than median annual BD exposures had higher MN values than lower exposed workers: frequency ratio (FR) of 1.30, 95% confidence interval (CI) 1.14–1.53; P < 0.05. Workers who carried the following genotypes were associated with greater frequency of MN (P < 0.05 for each comparison, unless specified): XRCC1 -77 CT genotype (FR = 1.28, 95% CI: 1.04–1.57; reference C/C), ADPRT 762 Ala/Ala (FR = 1.54, 95% CI: 1.17–2.03; P < 0.01), XRCC1 194 Arg/Trp (FR = 1.13, 95% CI: 0.87–1.27; reference, Arg/Arg), XRCC1 280 Arg/His (FR = 1.67, 95% CI: 1.10–2.42; reference, Arg/Arg), XRCC1 399 Arg/Gln and Gln/Gln genotypes (FR = 1.26, 95% CI: 1.03–1.53 and FR = 1.24, 95% CI 1.03–1.49; reference Arg/Arg, respectively). As XRCC1 polymorphisms were linked, workers carrying the XRCC1 (-77)-(194)-(280)-(399) diplotypes, TCGA/TGCA, had a higher MN frequency compared with individuals carrying the wild-type CCGG/CCGG (FR = 1.57, 95% CI: 1.02–2.41; P < 0.05). In conclusion, CB-MN is a sensitive index of early damage among BD-exposed workers. In workers exposed to BD, multiple BER polymorphisms and a XRCC1 haplotype were associated with differential levels of chromosome damage.

Introduction

1,3-Butadiene (BD) is an important industrial chemical used to manufacture synthetic rubber and other polymers. It is also a common air pollutant found in auto missions and cigarette smoke. Since the recognition of BD as a potent carcinogen in mice (1), BD has been the subject of intense research. The International Agency for Research on Cancer has classified BD as a Group 1 carcinogen (2). In recent years, BD exposure research has expanded to characterize its carcinogenic risk to humans and to understand the relationships between gene polymorphisms involved in BD exposure and the mechanisms of its toxicity leading to carcinogenicity (3). There is accumulating evidence that carcinogenic and mutagenic effects of BD are due to the formation of the epoxide metabolites, butadiene monooxepoxide, butadiene diepoxide and butadiene dioxepoxide (4). These metabolites form DNA adducts or protein adducts which result in genetic toxicity. Previous studies found that BD metabolites can induce genetic damage including an increased frequency of sister chromatid exchange (SCE), chromosome aberrations (CA) and micronuclei (MN). Some in vitro and animal experiments have demonstrated that the frequency of SCE and CA is increased in lymphocytes treated by butadiene monooxepoxide. An epidemiologic study in 19 BD exposure workers also found a high risk of CA and SCE (5,6); yet conflicting results were reported in another study, where there was no obvious change of SCE, MN or CA even when the BD exposure concentration was in the range of 2.2–7.4 mg/m³ (7). These conflicting results suggest that other factors, such as polymorphic variants in DNA repair genes, may modify the effect of BD on chromosome damage.

X-ray repair cross-complementing group 1 (XRCC1) gene products are involved in the repair of single-strand DNA breaks. The repair of single-strand DNA breaks arising directly from damage to the deoxyribose moieties or indirectly as intermediates of the base excision repair (BER) pathway (8) is facilitated by the scaffold protein XRCCI via its ability to interact with DNA ligase IIIα, DNA polymerase β, apurinic/apyrimidinic endonucleases (APE1), polynucleotide kinase/phosphatase and poly (adenosine diphosphate-ribose) polymerases 1 and 2 (ADPRT-1 and 2).

A major defense against alkylating mutations is provided by O6-methylguanine-DNA methyltransferase (MGMT), a DNA repair protein that transfers potentially carcinogenic O6-alkylation adducts from the DNA to a cysteine residue of MGMT (9,10). For each adduct removed, an MGMT molecule is inactivated. Single-nucleotide polymorphisms of the MGMT gene have also been associated with increased risks of cancer, especially among those exposed to alkylating mutagens.

The aim of the present study was to determine: (i) whether BD-exposed workers had increased chromosomal damage through CB-MN methods and (ii) whether common polymorphisms in the DNA repair pathway (XRCCI Arg194Trp, XRCCI Arg280His, XRCCI Arg399Gln and XRCCI -77C, MGMT Leu84Phe, ADPRT Val762Ala and APE1 Asp148Glu) modified the amount of chromosomal damage in BD-exposed workers.

Materials and methods

Study subjects

Workers from a Polybutadiene Latex (PBL) chemical industrial plant in Ningbo, China, were recruited during routine medical evaluations. Eligibility was based on employment records and was defined as occupational exposure to BD for at least 1 year. After informed consent was obtained, workers underwent an interviewer-administered questionnaire, which inquired about demographic characteristics, medical history, diet and smoking and drinking habits. A total of 166 Han Chinese workers, of whom 149 are males with a mean age and SD of 31.13 ± 6.72 years, and whom blood samples and completed questionnaires were available, were analyzed. In the case-control analysis, teachers and graduate students without BD exposure were selected as a control group, from the School of Public Health, Fudan University, and included 20 Han Chinese men and 21 women, with an average age of 35.31 ± 11.10 years. As the age and gender distributions between cases and controls were not identical, both age and gender were included as adjustment variable in all analyses.
A total of 10 ml anticoagulated peripheral blood was collected from each subject. Blood samples were stored at room temperature in an insulated container and were delivered to the laboratory within 12 h of collection. Each control subject and exposed worker completed a detailed questionnaire; cytokinesis-blocked (CB) MN assay tests were performed. Polymerase chain reaction (PCR) restriction fragment length polymorphism analyses (PCR) were performed on their blood samples.

Exposure assessments
Air samples. All PBL work sites involved in the production of butadiene, including operational industrial plant itself, product storage sites, waste water conduct and even administrative office, were selected for regular air sampling (once every 15 min). All data were collected through a local Center for Disease Control from 2002 to 2008. For each sampling location, two air samplers collected air samples once a day at a flow rate of 0.1 l/min for each sample (G栏Air-3 atmosphere sampler with constant flow; Gillian, Sensidyne company, Clearwater, FL). After sampling, each charcoal tube collected was desorbed in dichloromethane alkyl and the samples were analyzed with gas chromatograph (GC-14B, Shimadzu Corporation, Kyoto, Japan). The method depends on the determination of alkenes in the air of workplace (GBZ 160.39-2007).

DNA extraction and genotyping
Two milliliter of heparin-anticoagulated whole blood was collected from workers. DNA was extracted from peripheral lymphocytes using DNA extraction kits. DNA was frozen at −80°C. Approximately 50 ng of genomic DNA was amplified in GeneAmp 9600 (Perkin Elmer Corp., Waltham, MA) in a total volume of 15 μl consisting of 0.4 μl for each primer, 7.5 μl of 2 × PCR Mix and 5.7 μl of ddH2O.

Genetic polymorphisms were detected using the following primers: Arg194Trp—forward (F): 5'-GCCGCCACGTCCCAAGTA-3' and reverse (R): 5'-AGCCCCAAGACCCTTTCACT-3'; Arg84Leu/Leu—F: 5'-GAGAACGCTCTGTTACCTTAATGTCAGTTTT-3' and R: 5'-AGCCCCAAGACCCTTTCACT-3'; XRCC1 Arg194Trp—F: 5'-TTGGGGCTTCTGGTGGTCTCCTGTA-3' and R: 5'-CAGACCCACATACACACCAACCTGGAGG-3'; Arg399Gln—F: 5'-TTGGGGCTTCTGGTGGTCTCCTGTA-3' and R: 5'-TCTCCAGCCTTTTCTGATA-3'; XRCC1 Arg194Trp—F: 5'-TTGGGGCTTCTGGTGGTCTCCTGTA-3' and R: 5'-TCTCCAGCCTTTTCTGATA-3'; XRCC1 -77 T/C for 25 s and a final elongation step at 72°C for 10 min. For XRCC1 Arg194His, the same conditions were used except that the annealing temperature was 69°C; annealing temperatures varied for each polymorphism: it was 55°C for APE1, 57°C for ADPRF; 58°C for MGMT and 62°C for XRCC1 -77. Then the PCR products were digested at 37°C for 12 h with restricted endonucleases (Fermentas, Burlington, Ontario, Canada); MspI for XRCC1 Arg194Trp and XRCC1 Arg399Gln, Rsal for XRCC1 Arg194Trp, BsrBI for XRCC1 -77T/C, Bfai for APE1 Asp148Glu, Bstf for ADPRF Val762Ala and Hinfl for MGMT Leu84Phe. The PCR products of the XRCCL 194 Arg allele produced 346 and 37 bp bands; the Trp allele produced a 383 bp band. XRCCL 280 Arg allele exhibited a product fragment of 140 bp, whereas His alleles produced a 280 bp fragment. The XRCCL 399 Arg allele produced 277 and 186 bp bands and the Gln allele a single 463 bp band. XRCCL 1-77 allele produced 116, 57 and 46 bp bands and the C allele 173 and 46 bp bands. The APE1 148 Asp allele produced a single 165 bp product; the Gln allele, 146 bp. The ADPRF 76 Val allele produced a 156 bp band and the Ala allele 135 bp. MGMT 84 Leu/Leu produced a single 178 bp band and the Phe allele produced a 161 bp band.

Statistical methods
We assessed the statistical significance of tests for the Hardy–Weinberg equilibrium and linkage disequilibrium analysis using the method described by Shi et al. (12).

The risks of chromosomal damage associated with the genotypes were estimated by computing frequency ratios (FR = e0, e = 2.71828) and 95% confidence intervals (CI) from univariate and multivariate Poisson regression models with adjustments for age, sex, smoking status, alcohol drinking and cumulative BD exposure. For categorical variables, the FR indicated a proportional increase/decrease of the micronucleus frequency in a comparison group relative to the referent. All statistical analyses were done using the software SAS 9.1 (SAS Institute).

PHASE software (version 2.0.2) was used to obtain maximum-likelihood estimates of the XRCCLI haplotype frequencies.

Results
Exposure assessment
All areas of the industrial plant had detectable levels of butadiene; the PBL operational plant, specifically, had the highest concentration of all the sites. The transient concentration in PBL storage sites, operational sites and vibrating screen sites were 182.53, 198.59, 153.35 mg/m³, respectively. The values ranged from 0.05 to 1985.99 mg/m³.

Subject characteristics and risk estimates for demographic and lifestyle factors
Table I presents the study demographics and lifestyle characteristics of the workers, along with their associations with micronucleus frequency. The percentage of male subjects, current and former smokers and alcohol drinkers were 89.8, 56.6, and 25.3%, respectively. The mean cumulative BD exposure was 587 mg/year. There was a small increase in MN frequency among women and participants who were ≥35 years of age, but this did not reach statistical difference.

No significant difference in MN frequency was detected by smoking or alcohol drinking status. BD-exposed workers had a mean MN frequency of (3.39 ± 2.42) × 10−6 which was significantly higher than the mean MN frequency of the controls (1.48 ± 1.26) × 10−6 (P < 0.01). Within the workers themselves, Poisson regression demonstrated that high BD-exposed workers (>587 mg/year, where 587 mg/year was the median level of exposure) had a significantly increased MN frequency compared with the low BD-exposed group (≤587 mg/year; FR = 1.30, 95% CI: 1.14–1.53; P < 0.01).

Distribution of genotypes and risk assessment for genes polymorphism
The frequencies of APE1, ADPRF, MGMT and XRCCLI genotypes are found in Table II, and their association with chromosome damage (by CB-MN) in workers is presented in Table III. The minor (variant) alleles and their minor allele frequencies (in parentheses) were as follows: ADPRF T762A (26%), MGMT 84Phe (12%) and APE1 148Glu (37%). XRCC1 194 Trp (37%), XRCC1 280 His (17%), XRCC1 399 Gln (26%) and XRCC1 -77 C/T (16%). Genotype distributions at each locus were consistent with Hardy–Weinberg equilibrium. No associations with MN frequency were found with the MGMT Leu84Phe and APE1 Asp148Glu polymorphisms. In contrast, individuals carrying the ADPRF 762 Ala/Ala genotype had a significantly higher MN frequency, when compared with those carrying the Val/Val genotype (FR = 1.54, 95% CI: 1.17–2.03; P < 0.01). The XRCC1 -77 C/T genotype had a significantly higher MN frequency when compared with workers carrying the T/T genotype (FR = 1.28, 95% CI: 1.04–1.57; P < 0.05). There was a higher MN frequency for individuals who possessed XRCCLI 194 Arg/Trp genotypes compared with those carrying the Arg/Arg genotype (FR = 1.13, 95% CI: 1.07–1.27; P < 0.05). XRCCLI 280 His/His genotype was also associated with a significantly higher MN frequency, when compared with the Arg/Arg genotype (FR = 1.67, 95% CI: 1.10–2.42; P < 0.05). The XRCCLI 399 Arg/Gln genotype had a significantly higher MN frequency compared with those carrying Arg/Arg genotype (FR = 1.26, 95% CI: 1.03–1.53; P < 0.05).
Cumulative exposure to BD
Alcohol intake
Age (years)
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XRCC1 -77 C/T

Table IV presents the results of multivariate analysis using a backward stepwise selection of variables, including age, sex, smoking status, alcohol drinking, cumulative BD exposure and polymorphisms of XRCC1, APE1, ADPRT and MGMT. In the final model, subjects with XRCC1 -77 C/T, ADPRT 762 C, XRCC1 194 T, XRCC1 280 A and XRCC1 399 A variant alleles (based on a dominant genetic inheritance model) each had a statistically significantly higher MN frequency, when compared with their respective wild-type homozygous counterparts. Higher cumulative BD exposure was also associated with higher MN frequency.

Table II. Genotype and allele frequencies among BD-exposed workers

Table III. MN frequency among BD-exposed workers by DNA repair gene polymorphism

Multivariate Poisson regression analysis of MN frequency

Table I. MN frequency of BD-exposed workers and unexposed controls by various demographic characteristics

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Diplotypes of XRCC1 and MN frequency
To further elucidate the relevance of XRCC1 variants with MN frequency, linkage disequilibrium among the four XRCC1 polymorphisms (XRCC1 -77 C/T, Arg194Trp, Arg280His and Arg399Gln) was analyzed and haplotypes were reconstructed. The D' value of the four loci of XRCC1 were 0.821 (XRCC1 -77 with 194), 0.842 (XRCC1 -77 with 280), 0.862 (XRCC1 -77 with 399), 0.857 (XRCC1 194 with 280), 0.814 (XRCC1 194 with 399) and 0.803 (XRCC1 280 with 399). For all subjects, 28 XRCC1 (-77)-(194)-(280)-(399) diplotypes were identified in the analysis of the workers. The diplo
**Polymorphism and chromosomal damage induced by BD**

Due to the intrinsic instability of DNA and those that are induced by environmental chemicals. This study found that workers who were cumulatively exposed to BD at higher rates also faced a significantly higher risk of chromosome damage, when compared with less exposed workers and with unexposed controls. Among Chinese BD-exposed workers, the genotypes XRCC1 194 Arg/Trp, XRCC1 280 Arg/His, XRCC1 399 Arg/Gln, XRCC1 77 T/C and ADPRT 762Ala/Ala were each associated with increased MN frequency. In addition, workers carrying the XRCC1 TCGA/TCGA diplotype were also associated with greater chromosome damage.

Our exposure assessment data showed that PBL operational sites had the highest concentration. Similarly, Hayes et al. (13) measured exposure levels in a butadiene polymer production facility in China, the exposure range in polymer analysts was from below detection to 2382 mg/m³. Moreover, industrials exceeded exposure to butadiene in other countries were also reported (2).

Consistent with previous studies (14,15), our study found that there was no significant effect of smoking or alcohol drinking on MN frequency. The most plausible interpretation for this lack of association is that the magnitude of association with BD exposure was so strong that relationships with smoking or alcohol drinking were masked. Alternatively, blood concentrations of cigarette smoke or alcohol-related genotoxins may have been too low to cause chromosomal damage in lymphocytes (16). Previous epidemiologic studies have investigated the effect of various lifestyle and biological factors on MN frequency in human lymphocytes. The most consistent demographic variable influencing the MN frequency was age, with MN frequency increasing significantly with age (17). However, our results indicated that there was no significant increase in MN frequency among older workers compared with younger workers and no significant difference between female and male workers. A possible reason for these findings may be the limited number of older and female workers in this study.

There are >60 validated genetic polymorphisms in XRCC1 and ~30 variants are located in exons or promoter regions. The most extensively studied polymorphisms are Arg194Trp on exon 6, Arg280His on exon 9 and Arg399Gln on exon 10 (18). In our study, the exposed workers carrying XRCC1 194 Arg/Trp and Trp/Trp genotypes had statistically higher MN frequency, when compared with workers carrying the XRCC1 194Arg/Arg wild-type genotype. The XRCC1 194 Arg/Trp polymorphism is located in the XRCC1 nuclear localization signal domain, in proximity to other domains that mediate polymerase β and APE1 interactions (19). Therefore, this polymorphism may disturb XRCC1 protein conformation, resulting in a decreased protein affinity or decreased DNA damage binding and ineffective DNA repair. Zhu et al. (20) and Zhang et al. (21) reported that this polymorphism significantly decreased DNA damage repair in vinyl chloride monomer and benzene-exposed workers, respectively.

In our study, BD-exposed workers carrying the XRCC1 280 Arg/His and His/His genotypes were found to carry a higher chromosome damage than other who did not carry these genotypes. Previous research that investigated XRCC1 polymorphisms and their association with DNA repair capacity reported that only the variant proteins associated with the XRCC1 280 His allele is defective in the efficient localization of the protein to a damaged site in the chromosome, thereby reducing BER efficiency (22). Because XRCC1 Arg280His is located close to sequences that mediate protein–protein interactions with ADPRT and DNA polymerase β (23–25), this protein may be involved in the formation of unstable chromosomal aberrations (26).

Several studies (27–29) have found an association between the presence of the variant genotypes of the XRCC1 Arg399Gln polymorphism and reduced DNA repair capacity, including data showing increased MN frequency. Three studies (30–32) all reported a significant increase in CA frequency in the presence of a variant allele of the XRCC1 Arg399Gln polymorphism in benzene-exposed workers. However, others have reported no detrimental effect of variant XRCC1 399 Gln allele and XRCC1-mediated repair (33–36). In our study, worker exposed to BD and carrying the XRCC1 399 Arg/Gln and Gln/Gln genotypes had higher chromosome damage compared with workers carrying the Arg/Arg genotype.

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**Table IV.** Final multivariate Poisson regression analysis of the association between genetic polymorphism data (independent variable) and MN frequency (dependent variable)

<table>
<thead>
<tr>
<th>Name</th>
<th>β (95% CI)</th>
<th>P-value</th>
<th>FR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1.67 (1.11–2.21)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Cumulative BD exposure</td>
<td>0.11 (0.01–0.21)</td>
<td>0.0358</td>
<td>1.11 (1.01–1.14)</td>
</tr>
<tr>
<td>ADPRT 762</td>
<td>0.17 (0.08–0.27)</td>
<td>0.0062</td>
<td>1.19 (1.08–1.30)</td>
</tr>
<tr>
<td>XRCC1 -77</td>
<td>0.26 (0.12–0.50)</td>
<td>0.0285</td>
<td>1.30 (1.12–1.64)</td>
</tr>
<tr>
<td>XRCC1 194</td>
<td>0.16 (0.08–0.22)</td>
<td>0.0156</td>
<td>1.18 (1.08–1.25)</td>
</tr>
<tr>
<td>XRCC1 280</td>
<td>0.12 (0.02–0.21)</td>
<td>0.0175</td>
<td>1.12 (1.02–1.24)</td>
</tr>
<tr>
<td>XRCC1 399</td>
<td>0.14 (0.03–0.24)</td>
<td>0.0364</td>
<td>1.13 (1.04–1.27)</td>
</tr>
</tbody>
</table>

Because clinical characteristics (age, gender, smoking and alcohol status) were each non-significantly associated with MN frequency, these variables were not included in the final regression model. Because the frequency of homozygous variants for several of the polymorphisms was low, a dominant genetic inheritance model is presented. Cumulative BD exposure is a continuous variable. β, beta regression term in Poisson regression model.

**Table V.** Associations between diplotypes of XRCC1 and Micronuclei (MN) frequency

<table>
<thead>
<tr>
<th>Diplotype</th>
<th>n (%)</th>
<th>Mean MN ± SD</th>
<th>FR (95% CI)</th>
<th>Adjusted FR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCGG/CCGG</td>
<td>13 (9.4)</td>
<td>2.93 ± 2.43</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CCGA/CCGA</td>
<td>9 (6.5)</td>
<td>3.44 ± 2.83</td>
<td>1.14 (0.62–1.77)</td>
<td>1.17 (0.73–1.89)</td>
</tr>
<tr>
<td>TCGA/TCGA</td>
<td>10 (7.3)</td>
<td>4.60 ± 2.27</td>
<td>1.45 (1.00–2.37)</td>
<td>1.57 (1.02–2.41)</td>
</tr>
<tr>
<td>TCGA/TTGA</td>
<td>13 (9.4)</td>
<td>3.46 ± 2.63</td>
<td>1.15 (0.68–1.75)</td>
<td>1.18 (0.76–1.82)</td>
</tr>
<tr>
<td>CCCA/CTAG</td>
<td>8 (5.8)</td>
<td>2.63 ± 2.26</td>
<td>0.88 (0.51–1.41)</td>
<td>0.89 (0.52–1.51)</td>
</tr>
<tr>
<td>CCCA/CAGC</td>
<td>12 (8.7)</td>
<td>3.17 ± 2.55</td>
<td>1.12 (0.79–2.62)</td>
<td>1.08 (0.69–1.70)</td>
</tr>
<tr>
<td>CTCG/CTGG</td>
<td>16 (11.6)</td>
<td>2.87 ± 2.28</td>
<td>0.94 (0.61–1.46)</td>
<td>0.98 (0.64–1.51)</td>
</tr>
<tr>
<td>CCGG/CTGG</td>
<td>15 (10.9)</td>
<td>3.06 ± 2.69</td>
<td>1.07 (0.78–1.83)</td>
<td>1.04 (0.68–1.61)</td>
</tr>
<tr>
<td>OTHERS</td>
<td>42 (30.4)</td>
<td>3.07 ± 2.69</td>
<td>1.09 (0.71–1.73)</td>
<td>1.05 (0.73–1.53)</td>
</tr>
</tbody>
</table>

The diplotypes are defined as the allele present at positions -77(C/T), 194(C/T), 280(G/A) and 399(G/A), respectively. n, per thousand lymphocytes; OTHERS, group of all diplotypes with <5% frequency.

Multiple Poisson regression: FR adjusted by age, gender and cumulative exposure dose.

* P < 0.05.
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Arg399Gln and for the et al. ment with Brem the four polymorphisms of (41). Linkage disequilibrium analysis of the four polymorphisms of XRCC1 (-C77T, Arg194Trp, Arg280His and Arg399Gln) are in linkage disequilibrium, and this is in agreement with Brem et al. (38). Our results found a nominally higher FR for the XRCC1 TCGA/TCGA diplyotype (compared with the reference wild-type diplyotype) than with individual XRCC1 polymorphisms. Other studies have also suggested that XRCC1 haplotypes may be appropriate for assessing environment disease associations. For instance, Leng et al. (42) reported that XRCC1 haplotypes are associated with risk of chromosomal damage in Chinese coke-oven workers. Such a statistically significant association may be attributable to changes in XRCC1 function because the DNA repair capacity of mutant alleles was lower than that of wild-type alleles. However, no studies to date have confirmed that these diplyotypes are more strongly associated with altered XRCC1 activity than the individual polymorphisms. If confirmed, XRCC1 diplyotypes may be useful in future occupational health surveillance as markers to screen and monitor workers who are occupationally exposed to BD.

ADPRT1 is a nuclear protein that binds to DNA via zinc-finger motifs (43). ADPRT1-deficient mice were susceptible to carcinoma, when induced by certain gene toxic agents (44,45). The ADPRT1 polymorphism evaluated in this study, located within the COOH-terminal catalytic domain, has been associated with the risk of thyroid carcinoma, carcinoma of gastric cardia and smoking-related lung cancer (46,47). A functional study had reported that the at-risk variant allele exhibited a 40% reduction in enzyme activity that resulted in a subsequent reduction in BER capacity. This reduction in capacity may lead to genome instability associated with the increased risk of tumor development (48). Our study expands on these consistent results by reporting that the at-risk genotype in these other studies also correspond to higher MN frequency in BD-exposed workers, well before cancer development.

Genetic polymorphisms are common in the general population. Most polymorphisms are located outside gene boundaries and thus thought not to have any apparent effects on protein functions. If a polymorphism is within the coding region of a gene, amino acid substitution may occur and result in change of protein activity, whereas polymorphisms in the promoter will alter the efficiency of transcription and polymorphisms located at an intron or exon boundary in a gene may produce incorrect messenger RNA splicing, which results in incomplete or inactive proteins. Thus, in this study, although no association of polymorphisms in APE1 Asp148Glu and MGMT1 Leu84Phe with risk of BD exposed was found, other polymorphisms in the promoter and coding region of these genes, yet to be identified, may contribute to risk of BD exposure.

In conclusion, our results reported that BD-exposed workers had higher frequencies of MN compared with controls, a result that indicates a pronounced clastogenicity of the BD. The XRCC1 -777C, XRCC1 194 Arg/Trp, XRCC1 280 Arg/Gln, XRCC1 399 Arg/Gln, ADPRT 762 Ala/Ala genotypes and XRCC1 TCGA/TCGA diplyotype were each associated with higher levels of DNA damage among Chinese workers exposed to BD. These DNA repair polymorphisms results may have important implications for mechanisms of carcinogenesis. In order to understand better the mechanisms of BD-induced genotoxicity and ultimately carcinogenicity, it is important to evaluate additional variants of genes that participate in the metabolic pathway of BD. Particular attention should be paid to identify genetic markers as well as gene–gene and gene–environment interaction that may predict individual susceptibility to genetic damage.

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